Lanthanide Complexes with High Stability and Efficiency for the Hydrolysis of a Ribonucleotide Dimer

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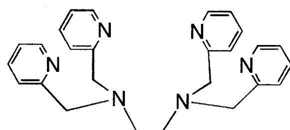
Lanthanide complexes with *N*,*N*,*N'*,*N'*-tetrakis[(2-pyridyl)-methyl]ethylenediamine (TPEN) efficiently hydrolyze a ribonucleotide dimer, ApA, at 35 °C, pH = 7.2. The activity of La^{3+}-TPEN is greater than that of free La^{3+} ion. The Ln^{3+}-TPEN complexes are sufficiently stable in neutral aqueous solutions (the formation constants are in the range log K/mol^{-1} dm^3 = 3.9 - 6.2). These complexes are promising for the active site of artificial ribonucleases.

Selective scission of RNA and DNA has been attracting much interest. The outstanding activity of lanthanide ions (Ln^{3+}) for the hydrolysis of phosphodiester bonds in RNA, DNA, and the related compounds have been reported.[1] Thus, it is strongly anticipated that the lanthanide complexes are useful for the active site of artificial nucleases. From this standpoint, some lanthanide complexes for the RNA hydrolysis have been investigated.[2] However, their hydrolysis activities are lower than those of the corresponding free ions. The stable coordination of the ligand seems to suppress the high activities of lanthanide ions in these complexes. For the molecular design of active site of artificial nucleases, both high stability and high hydrolysis activity are definitely required. Here we report that the lanthanide ions form stable complexes with *N*,*N*,*N'*,*N'*-tetrakis[(2-pyridyl)methyl]-ethylenediamine (TPEN),[3] which are sufficiently active for the RNA hydrolysis.



TPEN

Ln^{3+}-TPEN complexes show remarkable stabilities in aqueous solutions.[4] The formation constants of Ln^{3+}-TPEN complexes in aqueous solutions were determined at 446 nm by the competitive complex formation using 5-Br-PAPS (2-(5-bromo-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropylamino)-phenol).[5] The results are summarized in Table 1. The formation constants (log K/mol^{-1} dm^3) are in the range 3.9 - 6.2. In contrast, no evidence for the complex formation is detected for aliphatic polyamine ligands, i.e. TRIEN and TREN.[6] Significant roles of the pyridine moieties for the stable complex formation are strongly suggested.[7] Table 1 also lists the formation constants for the Ln^{3+}-IDA complexes, whose stabilities are relatively high due to the ionic interaction. The K values of TPEN complexes are only one or two orders of magnitudes smaller than those of IDA complexes. It is surprising that such high stabilities are observed for the Ln^{3+}-TPEN complexes although no ionic interaction participates in these complexes.[8]

Table 1. Formation constants (log K/mol^{-1} dm^3) of the Ln^{3+} complexes[a]

Ligand	La^{3+}	Pr^{3+}	Eu^{3+}	Ho^{3+}	Tm^{3+}	Lu^{3+}
TPEN	3.9	4.8	5.7	5.7	5.5	6.2
TRIEN	-	-	-	-	-	-
TREN	-	-	-	-	-	-
IDA	5.9	6.4	6.7	6.9	7.2	7.6

[a]pH=7.2, r.t. Chloride salts were used for all Ln^{3+}.
- : No evidence for the complex formation was observed by electronic absorption and ^{1}H NMR spectroscopies.[6]
TPEN = *N*,*N*,*N'*,*N'*-tetrakis[(2-pyridyl)methyl]ethylenediamine, TRIEN = triethylenetetramine, TREN = tris(2-aminoethyl)amine, IDA = iminodiacetate.

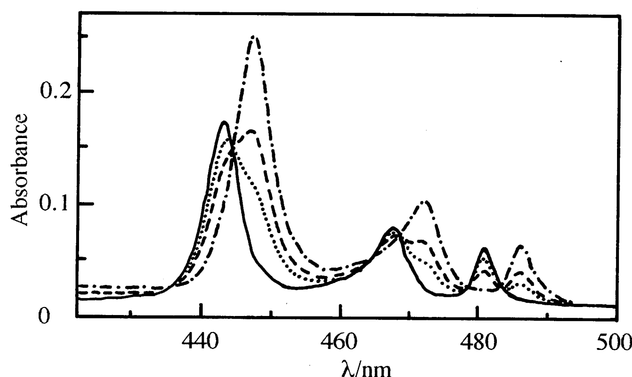


Figure 1. Electronic absorption spectra of the Pr^{3+}-TPEN mixture in H_2O, pH = 7.0. (a) $PrCl_3$ = 20 mmol dm^{-3}, TPEN = 0 mmol dm^{-3} (——); (b) $PrCl_3$ = 20 mmol dm^{-3}, TPEN = 5 mmol dm^{-3} (·····); (c) $PrCl_3$ = 20 mmol dm^{-3}, TPEN = 10 mmol dm^{-3} (----); (d) $PrCl_3$ = 20 mmol dm^{-3}, TPEN = 20 mmol dm^{-3} (-·-·-).

Table 2. Electronic absorption maxima of Pr^{3+} complexes (H_2O, pH 7.0)

Complex	λ/nm		
$PrCl_3$-TPEN	447.0	472.0	486.0
$PrCl_3$-TPA	444.0	469.5	484.0
$PrCl_3$-DPA	443.5	468.0	482.5
$PrCl_3$	443.0	467.5	480.5
assignment[9]	$^3P_2$	$^3P_1$	$^3P_0$

TPA = tris[(2-pyridyl)methyl]amine
DPA = bis[(2-pyridyl)methyl]amine

Table 3. Pseudo-first-order rate constants for the hydrolysis of ApA[a]

Compound	k/h^{-1}
$LaCl_3$	6.1 (± 1.0) $\times 10^{-3}$
$LaCl_3$-TPEN	9.2 (± 0.5) $\times 10^{-3}$
$LaCl_3$-IDA	-[b]
$PrCl_3$	1.3 (± 1.0) $\times 10^{-1}$
$PrCl_3$-TPEN	7.9 (± 0.9) $\times 10^{-3}$
TPEN	-[b]

[a] $[ApA]_0$ = 0.1 mmol dm^{-3}, $[Ln^{3+}]$ = 2 mmol dm^{-3}, [TPEN] or [IDA] = 4 mmol dm^{-3}, 35 °C, pH=7.2 (20 mM Hepes buffer). [b] Very slow (hydrolysis was negligible after 24 h).

Figure 1 shows the electronic absorption spectra of the Pr^{3+}-TPEN mixture. On the complex formation, absorption maxima in the visible region ($^3P_2$, $^3P_1$, and $^3P_0$)[9] shift to lower energy. The observation of independent peaks due to the complex and the free ion enables unambiguous assignment of these peaks (see Figure 1, b and c). The most part of Pr^{3+} forms the complex in the $[TPEN]/[Pr^{3+}] = 1$ mixture (Figure 1, d), which is consistent with the high formation constant of this complex (Table 1). The lower energy shifts of the absorption maxima strongly indicate the contribution of the 4f-orbitals to the complex formation, and π-electrons in the pyridine moiety should play the important role in the coordination.[10,11] Consistently, the magnitudes of the lower energy shifts depend on the number of pyridine moieties in the ligand (Table 2); the magnitudes are in the order TPEN > TPA > DPA for all peaks.

The TPEN-Ln^{3+} complexes are efficient for the hydrolysis of a ribonucleotide dimer, ApA. The pseudo-first-order rate constants at pH 7.2, 35 °C are summarized in Table 3.[12] It is particularly important that the La^{3+}-TPEN complex shows higher activity than that of the free La^{3+} ion (ca. 1.5 fold). The hitherto reported Ln^{3+} complexes have lower activities than the corresponding free ions.[2] The present results strongly indicate that much higher activity could be attained by more suitable design of the Ln^{3+} complex.

In conclusion, TPEN-Ln^{3+} complexes having great stabilities in aqueous solutions are efficient for the hydrolysis of the ribonucleotide dimer. Thus, the complexes of this type will be promising for the active site of artificial nucleases.[13]

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References and Notes

1 M. Komiyama, N. Takeda, Y. Takahashi, H. Uchida, T. Shiiba, T. Kodama, and M. Yashiro, *J. Chem. Soc., Perkin Trans. 2*, **1995**, 269; M. Komiyama, K. Matsumura, and Y. Matsumoto, *J. Chem. Soc., Chem. Commun.*, **1992**, 640; and references cited therein.

2 a) J. R. Morrow, L. A. Buttrey, V. M. Shelton, and K. A. Berback, *J. Am. Chem. Soc.*, **114**, 1903 (1992). b) K. O. A. Chin and J. R. Morrow, *Inorg. Chem.*, **33**, 5036 (1994). c) S. J. Oh, K. H. Song, and J. W.Park, *J. Chem. Soc., Chem. Commun.*, **1995**, 575.

3 G. Anderegg and F. Wenk, *Helv. Chim. Acta*, **50**, 2330 (1967). B. G. Gafford and R. A. Holwerda, *Inorg. Chem.*, **28**, 60 (1989).

4 E. Ohyoshi and S. Kohata, *J. Inorg. Biochem.*, **43**, 45 (1991).

5 The elementary analysis of the Pr^{3+}-TPEN complex (nitrate) indicates the 1:1 complex formation. Calcd for $(C_{26}H_{28}N_6)\cdot(NO_3)_3\cdot H_2O\cdot Pr$: C, 40.58; H, 3.93; N, 16.38%. Obsd: C, 40.92; H, 3.83; N, 16.37%.

6 The coordination of TRIEN or TREN to lanthanide ions in aqueous solutions is so weak that their complex formation has been studied in non-aqueous media (J. H. Forsberg, "Gmelin Handbuch der Anorganischen Chemie, 8th ed., Rare Earth Elements Part D1," ed by T. Moeller and E. Schleitzer-Rust, Springer-Verlag, Berlin, Heiderberg, New York (1980), Chap. 2).

7 ^1H NMR spectroscopy (270 MHz, D_2O, pD 7) indicates the slow ligand exchange of the La^{3+}-TPEN complex. Signals due to the La^{3+}-TPEN complex and free TPEN are observed independently at r.t. At the higher temperature (50 °C), broadening of signals is observed due to the acceleration of the ligand exchange. The formation constants of the La^{3+}-TPEN and La^{3+}-TPA complexes estimated from the ^1H NMR spectra are ca. log K = 3 and 2, respectively.

8 The formation constants of Ln^{3+}-TPEN complexes are much higher than those of Ln^{3+} complexes with octadentate aminoalcohol ligands[2b] (K/mol^{-1} dm^3 = 62 - 130). Superiority of TPEN as a ligand is strongly suggested.

9 L. I. Katzin and M. L. Barnett, *J. Phys. Chem.*, **68**, 3779 (1964).

10 L. C. Thompson, *Inorg. Chem.*, **3**, 1015 (1964). K. Tatsumi, K. Kasuga, and M. Tsutsui, *J. Am. Chem. Soc.*, **101**, 484 (1979).

11 This is further supported by the result that no shift was observed with TREN (even with the large excess), an aliphatic amine analog of TPA.

12 Hydrolysis of ApA was followed by a reversed phase ODS HPLC, detected by absorption at 258 nm. The products are adenosine, its 2'- and 3'-phosphates, and the 2',3'-cyclic phosphate, as usually observed in the metal assisted hydrolysis of ApA.[1] The reaction of UpU with the $LaCl_3$-TPEN complex (k = 5.7 x 10^{-3} h^{-1}) yielded also only the hydrolysis products.

13 These complexes are expected to exert sufficiently high activities in the artificial RNase constituting of the Ln^{3+} complex and DNA which is complementary to the target RNA, because the concentrations of intramolecular catalysts are in general equivalent to about 10 mol dm^{-3} intermolecular catalysts (M. L. Bender, "Mechanisms of Homogeneous Catalysis from Protons to Proteins," Wiley Interscience, New York, p. 643 (1971)).